obesity, hypertension, joint involvement, tophus formation, serum uric acid, and renal failure.

The patients all formed a homogeneous clinical group. An exception to this must lie on the matter of uric acid stone formation, since it has been demonstrated that heavy excretion is an important predisposing factor here (Yü & Gutman 1967). Our own numbers were too small for a valid comparison to be made. Again, our remarks do not apply to patients with gout secondary to blood dyscrasias, who tend to have a high excretion.

The well-known familial aspects of gout and hyperuricæmia have prompted a number of family and epidemiological investigations. It is evident from what has been said that such studies can have only a limited value, since numerous causative mechanisms are operating. Population studies have mostly tended to show roughly normal distribution of serum urate values in the two sexes. Among numerous family studies, one of some significance was that of Hauge & Harvald (1955), who compared serum urate values in the siblings of gouty patients with those of a control group. Values were significantly higher in the former. Moreover, serum urate values in the siblings could not be fitted to any mendelian pattern of inheritance, but fell into a normal distribution curve, suggesting cumulative or multifactorial influences.

We have recently examined the excretion of uric acid in first-degree relatives of patients with gout (Scott 1969), and it was found that a rough but significant graded correlation existed between the level of urate clearance in the gouty probands and the mean clearance of their relatives. It seems therefore that the concept of multifactorial influences applying to the level of uric acid in the blood may be extended to the handling of urate by the kidney, as expressed by urate clearance. With regard to the relative importance of genetic as opposed to environmental influences we have little information, but the recent study by Boyle *et al.* (1967) of intrapair variances in monozygotic and dizygotic twins indicated a strong genetic component determining serum uric acid levels in females; this was not detected in males, and the significance of this sex difference remains uncertain.

Further progress undoubtedly lies in the continued clarification of the many individual factors regulating uric acid metabolism. On the evidence I have presented it may be thought that hyperuricæmia in most instances will eventually be shown to result from a quantitative variation in a large number of these factors, rather than from single major abnormalities of the type exemplified by the Lesch-Nyhan syndrome.

A further problem then lies in the relation of hyperuricæmia to crystal deposition and the development of clinical gout, and this has yet to be explored in detail.

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Hyperuricæmia: Some Biochemical Aspects

The serum uric acid level depends upon a balance between the rate of *de novo* purine synthesis and nucleoprotein turnover, and uric acid disposal by renal excretion, by the gastrointestinal route, in sweat and by nonuricase mediated uricolysis (Howell & Seegmiller 1962).

The purine ring is assembled from small molecular precursors by nine enzymatic reactions, beginning with formation of ribofuranosylamine-5-phosphate from 5-phosphoribosyl-1-pyrophosphate ('PR-PP') and glutamine and ending with the production of hypoxanthine ribonucleotide (inosinic acid). The biochemistry of this fundamental metabolic pathway has been extensively reviewed with particular reference to clinical problems by Seegmiller, Laster & Howell (1963), Gutman & Yü (1965), Wyngaarden (1965, 1966) and Watts (1966). The purine ribonucleotide interconversion reactions and the catabolic pathways which lead to uric acid are summarized in Fig 1.

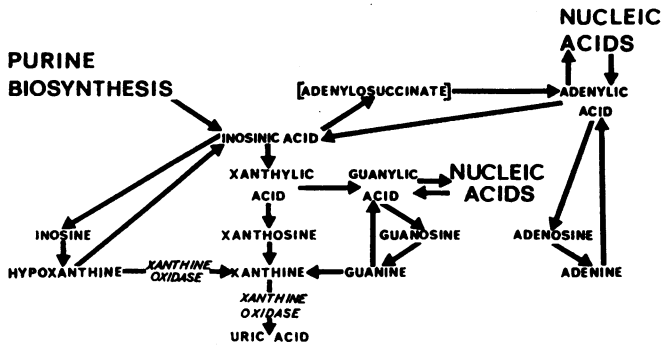

Fig 1 *The purine ribonucleotide interconversion reactions and the metabolic pathways which lead to uric acid. (Reproduced from Watts 1966)*

The methods which are now used for the detailed investigation of purine metabolism and uric acid production in man have been fully reviewed by Seegmiller (1967). Precise studies of urate production and of the renal handling of uric acid require that the subject shall have taken a repetitive purine-free diet for five days before as well as during the investigation, and that a specific enzymatic method of measuring uric acid is employed (Liddle *et al.* 1959). Dietary purine restriction is not needed in the routine clinical investigation of patients with gout, but the value of using a specific enzymatic method of measuring uric acid merits more widespread attention, especially if determinations of the urinary excretion of uric acid are needed.

The simultaneous determination of the renal clearances of inulin and uric acid in conjunction with evaluation of the rate of *de novo* purine synthesis by studying the incorporation of isotopically labelled glycine into the urinary uric acid (*vide infra*) showed that in some patients with gout the hyperuricæmia was due to reduced urate excretion. In others, uric acid production was excessive, and both defects were present in a third group of hyperuricæmic patients (Seegmiller *et al.* 1961).

Benedict *et al.* (1949) introduced the use of isotopically labelled uric acid to measure the size of the miscible urate pool and its turnover rate. Normal values are significant, but the increased values for the pool size, which are observed in gout, lack quantitative value because urate deposits equilibrate slowly with the injected labelled uric acid. This produces a progressively enlarging miscible urate pool, and an erroneously high value for the turnover rate. The study of the time course of labelling and the cumulative incorporation of a labelled small molecular urate-precursor, which is usually glycine, into the urinary uric acid has enabled some hyperuricæmic patients to be classified as 'over-producers' of uric acid and permitted the effect of investigative procedures on the rate of *de novo* purine synthesis to be studied. In these studies, a single dose of isotopically labelled (e.g. ^{14}C) glycine is given by mouth simultaneously with an intravenous dose of uric acid labelled with another isotope (e.g. ^{15}N). The latter enables the uric acid pool size and turnover rate to be evaluated; and, if the urinary uric acid content is known, the fraction of the uric acid turnover which is excreted can be calculated. A direct correction can also be made for loss of isotope due to the extrarenal disposal of uric acid synthesized from labelled glycine. The abnormally rapid increase in the urine uric acid labelling from glycine and the excessive cumulative incorporation of isotope from this source which characterizes the subjects who are over-producers of uric acid have been described by several groups of workers, and their results were summarized by Wyngaarden (1965).

Seegmiller *et al.* (1955) studied the role of 4-amino-5-imidazolecarboxamide (AIC) in purine biosynthesis in man, and observed that, although this compound was a uric acid precursor, it also reduced the incorporation of isotopically labelled glycine into the urinary uric acid. They interpreted this as evidence that AIC slowed *de novo* purine biosynthesis, presumably by inhibiting the first specific step of the purine biosynthetic pathway. Biochemical feedback control mechanisms are known to operate at this point in biosynthetic sequences (*see* for example Wyngaarden *et al.* 1958, Martin 1963), and the hypothesis was developed that, in the over-producer hyperuricæmic patients, this step is subject to a subnormal degree of feedback inhibition by the end-products of purine ribonucleotide biosynthesis. Wyngaarden & Ashton (1959) showed that avian phosphoribosylamidotransferase (EC 2.4.2.14) is in-

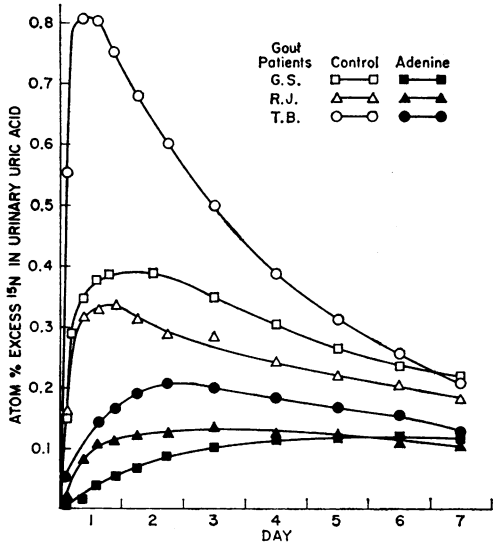

Fig 2 *Effect of adenine on the incorporation of* [^{15}N] *glycine into urinary uric acid in patients with gout.* (*Reproduced from Seegmiller* et al. *1968, by kind permission*)

hibited by purine ribonucleotides as well as by AIC-ribonucleotide, and Jones *et al.* (1962) demonstrated increased metabolic turnover of 5-phosphoribosyl-1-pyrophosphate in certain gouty subjects.

The rate of *de novo* purine biosynthesis, as judged by the incorporation of labelled glycine into urinary uric acid can be modified. It is reduced by, for example, the glutamine analogue 6-diazo-5-oxo-L-norleucine (Grayzel *et al.* 1960) and increased by 2-ethylamino-1,3,4-thiadiazole (Seegmiller *et al.* 1959, Seegmiller, Grayzel, Liddle & Wyngaarden 1963).

Seegmiller *et al.* (1968) showed that adenine administration reduces *de novo* purine synthesis in normal subjects and in patients with gout and excessive uric acid production. These workers used [^{15}N] glycine to assess *de novo* purine synthesis (Fig 2), [2-^{14}C] uric acid to correct for the extrarenal disposal of uric acid and to study the urate pool size and turnover rate, and [8-^{13}C] adenine to demonstrate that uric acid derived from adenine masked the expected effect of decreased uric acid biosynthesis on the total uric acid excretion (Fig 3).

The bond between the number nine nitrogen atom of the purine ring and the ribose moiety of the ribonucleotides is formed in the first reaction which is specific to purine biosynthesis (i.e. the phosphoribosylamidotransferase (EC 2.4.2.14) reaction). The subsequent reactions which lead to inosinic acid, as well as the purine ribonucleotide interconversion reactions (Fig 1) which form adenylic and guanylic acids, leave this bond intact. Free purines derived from the diet or produced from ribonucleotides by hydrolytic and phosphorolytic reactions have to be converted to ribonucleotides by the phosphoribosyltransferase enzymes before they can enter the main stream of purine metabolism and contribute to adenylic or guanylic acid production, or affect the rate of the phosphoribosylamidotransferase reaction. The phosphoribosyltransferase enzymes are: (1) AMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7) which converts adenine to adenylic acid; it also acts on AIC to form AIC-ribonucleotide. (2) IMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.8) which converts hypoxanthine to inosinic acid (IMP), guanine to guanylic acid (GRP) and 6-mercaptopurine to its ribonucleotide.

A phosphoribosyltransferase which converts xanthine to xanthylic acid (XRP) was recently reported by Kelley, Rosenbloom, Henderson & Seegmiller (1967*a*). These enzymes are present in blood cells and liver tissue, and the observation that the blood cells of patients with sex-linked essential hyperuricosuria (Lesch & Nyhan 1964) lack IMP:pyrophosphate phosphoribosyltrans-

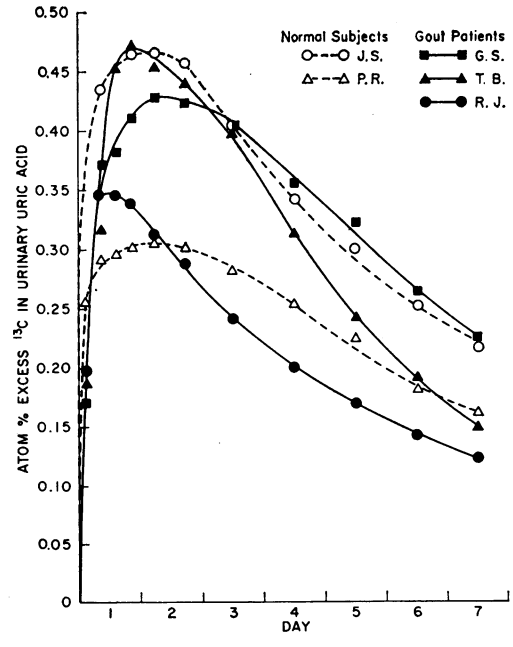

Fig 3 *The incorporation of* [8-^{13}C] *adenine into urinary uric acid in normal subjects and gouty patients.* (*Reproduced from Seegmiller* et al. *1968, by kind permission*)

ferase (Seegmiller *et al.* 1967) was a major advance which has greatly increased our understanding of the metabolic abnormalities in some cases of hyperuricæmia. Patients with this condition (the 'Lesch-Nyhan Syndrome') present with choreo-athetosis, mental retardation and compulsive self-mutilation in early childhood, having been normal at birth and in early infancy. Shapiro *et al.* (1966) and Nyhan *et al.* (1967) studied the genetics of the disease, and the mosaicism of the heterozygous female carriers was demonstrated by Rosenbloom, Kelley, Henderson & Seegmiller (1967) in conformity with the Lyon hypothesis of random inactivation of one member of each pair of X- chromosomes in females (Lyon 1961).

Studies with isotopically labelled glycine have shown that purine biosynthesis is greatly increased in patients with the Lesch-Nyhan syndrome and the clinical course of the condition is complicated by uric acid urolithiasis (Howard & Walzak 1968) and less frequently by acute gouty arthritis. The neurological manifestations dominate the clinical picture. Rosenbloom, Kelley, Miller, Henderson & Seegmiller (1967) showed that IMP:pyrophosphate phosphoribosyltransferase was lacking from the basal ganglia of a fatal case. These workers also reported that the cerebrospinal fluid contains excessive amounts of hypoxanthine and xanthine, and Balis *et al.* (1967) reported increased urinary excretion of these oxypurines in the disease. The presence of increased amounts of the oxypurines reflects the greatly increased rate of *de novo* purine synthesis. Azathioprine does not reduce purine biosynthesis in the Lesch-Nyhan syndrome because the drug must be converted to its ribonucleotide before it can exert a pseudo-feedback inhibitory action on the phosphoribosylamidotransferase reaction (Kelley, Rosenbloom & Seegmiller 1967, Sorensen & Benke 1967, Nyhan, Sweetman, Carpenter, Carter & Hafnagel 1968). Similarly, although allopurinol reduces the blood and urine uric acid levels and increases the oxypurine concentration further by inhibiting xanthine oxidase, it does not reduce the total purine production (i.e. the sum of the uric acid, hypoxanthine and xanthine excretions), because its pseudo-feedback inhibitory activity is also referable to the ribonucleotide derivative and not to the free drug. The final diagnosis of the Lesch-Nyhan syndrome depends upon demonstrating the deficiency of erythrocyte IMP:pyrophosphate phosphoribosyltransferase, and relatively simple methods of doing this have been described by Fujimoto *et al.* (1968) and by Berman *et al.* (1968). The use of the urinary uric acid to creatinine ratio as a screening test for inherited disorders of purine metabolism has also been suggested (Kaufman *et al.* 1968).

The erythrocyte IMP:pyrophosphate phosphoribosyltransferase activity has been measured in only a few patients with classical gout. Kelley, Rosenbloom, Henderson & Seegmiller (1967*b*) reported two kindreds in which sibs had reduced levels of IMP:pyrophosphate phosphoribosyltransferase. Although the enzyme activity was greatly reduced in these patients it was not completely absent as in the Lesch-Nyhan syndrome. Uric acid nephrolithiasis had occurred in childhood in both families and had been accompanied by attacks of acute gouty arthritis in one of them. The members of one family had the signs of a mild spinocerebellar disorder, whereas the members of the other family were neurologically normal. The families were also different biochemically in that the abnormal enzymes differed in their relative activity for each of the natural purine substrates and in their heat stability. The activity of adenine: pyrophosphate phosphoribosyltransferase was increased in all of these patients, but it is not known if this should be regarded as being in any sense a 'compensatory phenomenon'.

Kelley, Rosenbloom, Miller & Seegmiller (1968) extended the observation that allopurinol did not reduce total purine production, although it inhibited xanthine oxidase in patients with Lesch-Nyhan syndrome, to patients with excessive purine production and gout. They showed in a small series of 11 patients that those whose total purine production was reduced by allopurinol had normal IMP:pyrophosphate phosphoribosyltransferase activity, whereas the enzyme was deficient in the patients whose total purine production was unaltered by allopurinol.

The congenital absence of AMP:pyrophosphate phosphoribosyltransferase from blood cells was reported by Kelley, Levy, Rosenbloom, Henderson & Seegmiller (1968). This abnormality is not associated with excessive uric acid production. It is possible that more than one isoenzyme exists, and that only the blood cell enzyme is deficient.

2-ethylamino-1,3,4-thiadiazole stimulates purine production in patients with the Lesch-Nyhan syndrome (Nyhan, Sweetman & Lesch 1968), indicating that this compound does not act on purine biosynthesis by inhibiting IMP: pyrophosphate phosphoribosyltransferase. This agrees with the observation that 2-ethylamino-1,3,4-thiadiazole does not inhibit any of the blood cell phosphoribosyltransferase enzymes *in vitro* (Dean, Watts & Westwick, unpublished data).

Hooft *et al.* (1968) reported a case of hyperuricosuric encephalopathy without hyperuricæmia. The patient was a 3-year-old retarded girl whose parents were first cousins. She displayed self-mutilation, choreo-athetosis, and increased incorporation of isotopically labelled glycine into

urinary uric acid. The activity of her blood cell purine pyrophosphate phosphoribosyltransferase enzymes was not determined, but the sex of the patient indicates that the biochemical lesion is different from the one which characterizes the Lesch-Nyhan syndrome.

Fibroblast cultures have been useful in some recent studies on the control of purine biosynthesis in human tissues. Thus, cell lines cultured from patients with the Lesch-Nyhan syndrome (i.e. complete absence of IMP:pyrophosphate phosphoribosyltransferase) show acceleration of the early steps of the purine biosynthetic pathway, although the first specific step, the phosphoribosylamidotransferase reaction cannot yet be studied separately from the second step of the pathway. There is accumulation of 5-phosphoribosyl-1-pyrophosphate, although the rate of synthesis of this compound is not increased, and purine synthesis in the cells is sensitive to feedback inhibition by adenine ribonucleotides and 6-methylmercaptopurine ribonucleoside. Hypoxanthine and guanine do not inhibit, and in fact increase, the rate of purine biosynthesis, and the catabolism of newly synthesized inosinate is increased in the enzyme-deficient cells (Rosenbloom, Henderson, Caldwell, Kelley & Seegmiller 1968). Similar but less marked changes were reported in cell lines derived from patients with gout who had a partial deficiency of IMP: pyrophosphate phosphoribosyltransferase (Rosenbloom, Henderson, Kelley & Seegmiller 1968, Henderson, Rosenbloom, Kelley & Seegmiller, 1968).

It should be emphasized that a clear-cut abnormality of enzyme activity has so far been demonstrated only in a minority of hyperuricæmic patients, even among those with evidence of excessive purine biosynthesis. The nature of the abnormality in terms of the structure of the enzyme protein is also unknown. Henderson, Brox, Kelley, Rosenbloom & Seegmiller (1968) studied the enzyme kinetics of normal human blood cell IMP:pyrophosphate phosphoribosyltransferase, and similar studies of AMP:pyrophosphate phosphoribosyltransferase were made by Dean, Watts & Westwick (1968). The latter workers noted that phosphoribosylpyrophosphate inhibited the reaction under some conditions.

It is to be anticipated that further detailed enzymological studies will demonstrate new biochemical abnormalities and possibly more examples of partial defects which could accelerate *de novo* purine synthesis. It seems likely that the hyperuricæmic population may prove to be as heterogeneous in terms of biochemistry as it is in clinical manifestations.

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